

Structure of the Arabinogalactan from *Zea* Shoots¹

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ABSTRACT

The structure of the arabinogalactan obtained from the buffer-homogenate of *Zea mays* L. (hybrid B73 × Mo17) shoots has been studied. The purified polysaccharide was investigated by methylation analysis before and after controlled acid hydrolysis. Arabinogalactan-1 consists of arabinose, galactose, xylose, uronic acid, and glucose in the molar ratio of 37.1:55.8:3.0:4.1:trace, and arabinogalactan-2 consists of the same sugars in the ratio of 35.4:53.9:1.6:9.2:trace. A trace of protein was detected in arabinogalactan-1 and about 0.2% was present in 2. About 20% of the galactose residues in arabinogalactan-1 constitute a (1 → 3)-linked galactan chain and approximately 60% constitute a (1 → 6)-linked galactan sequence. About 15% of the galactose residues in arabinogalactan-1 are substituted by galactose in the 3- and 6-positions, thereby constituting branch points of the galactan framework. The remainder (5%) of the galactose residues in arabinogalactan-1 are located at nonreducing terminal positions. About 85% of the (1 → 6)-galactosyl sequence is substituted, mostly by single arabinose residues. Nonreducing terminal glucuronic acid is attached to C-6 of galactose residues. The basic structure of arabinogalactan-2 is similar to that of arabinogalactan-1.

The monocotyledonous coleoptile has been widely used as an experimental material in investigation of cell elongation mediated by a plant hormone, IAA. A number of studies have focused on the monosaccharide composition of various kinds of coleoptile cell walls and on the changes in their composition during growth (2, 6, 14, 16, 18, 19, 22-24, 26, 30, 33). Although information is available regarding structures of some components of polysaccharides in the growing monocot cell walls (7, 10-13, 15, 20, 21, 28, 31, 32), the complete structure of cell wall polysaccharides of any growing monocot has not been elucidated. To obtain detailed structural features of cell walls in growing monocots, we have examined *Zea* shoots which were excised at the coleoptile node 96 h after imbibition. Structural analyses were conducted on soluble polysaccharides obtained in the course of preparation of cell walls and on certain components released from isolated *Zea* shoot cell walls upon treatment with enzymes. This paper is the first in a series dealing with the polysaccharides of *Zea* shoots and describes the structure of arabinogalactan.

MATERIALS AND METHODS

Plant Material. Imbibed corn caryopses (*Zea mays* L., hybrid B73 × Mo17; Brayton Seed Co., Ames, IA) were soaked in running water for 2 h, sown on moist vermiculite, and maintained at 26°C under darkness. After 96 h, entire seedling shoots above the coleoptile node were excised and stored at -20°C.

Preparation of *Zea* Shoot Cell Walls and Water-Soluble Pol-

ysaccharide Fractions. Approximately 850 g of seedling shoots were homogenized in 2 L of ice-cold 0.3% NaCl in 10 mM Na-phosphate buffer (pH 6.5) for 5 min using a Waring Blender. The homogenate was filtered through premoistened Miracloth. The insoluble material was washed twice with 2 L of ice-cold 0.3% NaCl in 10 mM Na-phosphate buffer (pH 6.5). The combined filtrate and washings was made 5% with respect to TCA and after 10 h at 0°C was centrifuged for 30 min. The supernatant was dialyzed against distilled H₂O, concentrated to an appropriate volume, and freeze-dried to give WSP-I.²

The cell wall material recovered from the homogenate was suspended in 200 ml of 3 M LiCl in 50 mM McIlvaine buffer (pH 6.8) and maintained for 48 h at 4°C. Then the suspension was filtered through Miracloth and washed with a small volume of the same buffer. The combined filtrate and washing were heated in a boiling water bath for 10 min, dialyzed against distilled H₂O, and finally freeze-dried to give WSP-II.

The LiCl-treated cell wall material suspended in water was heated in a boiling water bath for 15 min and filtered through Miracloth. Three volumes of methanol were added to the filtrate. Precipitated material was recovered by centrifugation, washed successively with methanol and acetone, and dried *in vacuo* to give WSP-III.

The cell wall material obtained by this procedure was freeze-dried to give *Zea* shoot cell walls.

Resolution of WSP-I by Chromatography on DEAE-Sephadex A-25. WSP-I (400 mg) was dissolved in 10 ml of 20 mM Na-phosphate buffer (pH 6.0) and centrifuged. The supernatant was applied to a column (2.1 × 30 cm) of DEAE-Sephadex A-25 (Pharmacia Fine Chemicals) pre-equilibrated with the same buffer, and then the column was washed with the buffer. The adsorbed materials were eluted with a salt gradient (0-0.8 M in Na-phosphate buffer) followed by elution with 0.5 N NaOH. Seventeen-ml fractions were collected. Each tube was assayed for carbohydrate and protein. Tubes 3 to 5, 18 to 20, 21 to 26, and 27 to 40 were separately combined, dialyzed against distilled H₂O, and freeze-dried. Tubes 3 to 5 and 21 to 26 were designated WSP-I-1 and WSP-I-2, respectively.

Purification of WSP-I-1 and WSP-I-2 by Chromatography on Bio-Gel A-1.5m. WSP-I-1 (30 mg) was dissolved in 2 ml of 20 mM Na-phosphate buffer (pH 6.0) and centrifuged. The supernatant was applied to a column (2.2 × 52 cm) of Bio-Gel A-1.5m (100-200 mesh, Bio-Rad) pre-equilibrated with 20 mM Na-phosphate buffer (pH 6.0) followed by filtration with the same buffer; 0.9-ml fractions were collected. Each tube was assayed for carbohydrate and protein. Tubes 79 to 91 were combined, dialyzed against distilled H₂O, and freeze-dried to give purified WSP-I-1 (AG-1). WSP-I-2 (20 mg) was chromatographed under the same conditions. Tubes 82 to 95 were combined, dialyzed against distilled H₂O, and freeze-dried to give purified WSP-I-2 (AG-2).

Partial Acid Hydrolysis of AG-1. AG-1 (11 mg) was hydro-

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² Abbreviations: WSP-I, -II, and -III, water-soluble polysaccharide fractions, I, II, and III; AG, arabinogalactan.

lyzed under reflux with 2 ml of 0.05 N HCl in a boiling water bath for 30 min, and the hydrolysate was poured into 2 volumes of methanol and mixed. The mixture was maintained at 4°C overnight and then centrifuged. The precipitate was washed successively with 66% methanol, methanol and ether, and dried to give partially degraded AG-1 (AG-1-a). The combined supernatant and washings was air-dried to give fragments of AG-1 (AG-1-b).

Hydrolysis of AG-1-a with Acid and Fractionation of the Hydrolysate. AG-1-a (2.3 mg) was hydrolyzed under reflux with 1 ml of 0.1 N HCl in a boiling water bath for 2.5 h. After hydrolysis, the solution was neutralized with 0.1 N NaOH. The neutralized solution was applied to a column (Bio-Gel P-2, 1.5 × 150 cm), followed by filtration with water at 50°C; 1-ml fractions were collected and assayed for carbohydrate.

Analysis of Neutral Sugars in Polysaccharides. Polysaccharide (0.1–1 mg) was hydrolyzed with 2 N H₂SO₄ for 5 to 6 h at 100°C. After the hydrolysis, *myo*-inositol was introduced into the hydrolysate. The hydrolysate was then neutralized with barium carbonate, treated with Dowex 50W-X8 (H⁺ type, J. T. Baker Chemical Co.), and concentrated. Sugars converted to their respective alditol acetates were analyzed by GLC on a glass column (0.2 × 190 cm) packed with 3% SP-2340 (Supelco) (column a) at 210°C at a helium flow rate (35 ml/min).

Methylation Analysis of Polysaccharides. Polysaccharide (0.2–1 mg) in dimethyl sulfoxide (0.5 ml) was methylated with methyl sulfinyl carbanion (0.5 ml) and methyl iodide (0.2 ml) by the method of Hakomori (9) as described by Sandford and Conrad (27). Methylation of polysaccharide was performed three times. The methylated polysaccharide was hydrolyzed with 90% HCOOH, 0.5 N H₂SO₄ (17). The hydrolysate was neutralized with barium carbonate, deionized with Dowex 50W-X8 (H⁺ type) and concentrated to dryness. The methylated sugars were analyzed by GLC on a 10-m SP-2330 glass WCOT capillary column (Supelco) (column b) or a 30-m DB-1 fused silica capillary column (J & W Scientific Inc.) (column c) at 150 to 230°C with a split ratio of 50:1 as their corresponding alditol acetates (17). The helium carrier flow was 1.0 ml/min. For GLC-MS, a combined gas chromatograph-mass spectrometer, Finnegan 4000 with INCOS data system, fitted with a DB-1 fused silica capillary column (25-m) was used.

Methylation Analysis of the Acidic Oligosaccharide. Oligosaccharide (50 µg) was methylated by the method of Hakomori (9). The methylated oligosaccharide was reduced with LiAlH₄ according to the procedure of Sandford and Conrad (27). The carboxyl-reduced methylated oligosaccharide was remethylated by the method of Hakomori. The fully methylated neutral sugar thus prepared was hydrolyzed and converted into corresponding alditol acetates. Analysis of the derivatives was by GLC as described above.

General Methods. Concentration of carbohydrate solutions by rotary evaporation was performed under diminished pressure at 35 to 40°C. Paper chromatography was performed on Whatman filter paper No. 1 by the multiple ascending method with butanol:pyridine:water (6:4:3, v/v). Neutral sugars on the chromatogram were detected with alkaline silver nitrate (25). GLC was performed with a Varian model 3700 gas chromatograph. Protein was estimated using the Bio-Rad protein assay with BSA employed as standard. Total carbohydrate was determined by the phenol-H₂SO₄ method (8) and uronic acid was by the carbazole-H₂SO₄ method (3).

RESULTS

Isolation of Arabinogalactans. The preparation procedures of water-soluble polysaccharide fractions and cell wall materials from *Zea* shoots are summarized in Figure 1. A buffer-homogenate of *Zea* shoots was separated into soluble and insoluble

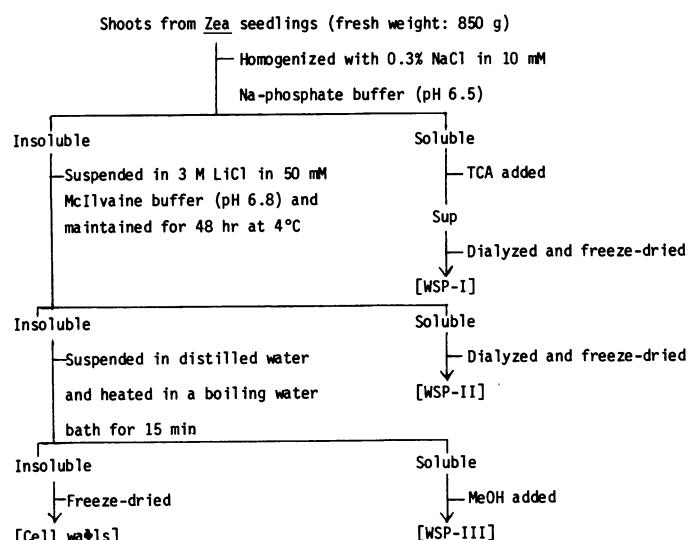


FIG. 1. A summary of preparation procedures of water-soluble polysaccharide fractions and cell wall materials from *Zea* shoots. Each step was performed as described under "Materials and Methods."

Table I. Yields, Total Carbohydrate Content, and Neutral Sugar Composition of Water-Soluble Polysaccharide Fractions I, II, and III

Fraction	Yield ^a	Total Carbohydrate Content ^b	Neutral Sugar Composition				
			Ara	Xyl	Man	Gal	Glc
	mg	mg	mol%				
WSP-I	416	208	32.1	5.7	0.8	48.5	12.8
WSP-II	440	339	4.8	3.1	0.0	3.1	89.0
WSP-III	330	202	2.1	1.1	0.1	2.1	94.6
Cell walls	13,300	ND ^c					

^a From 850 g of *Zea* shoots (fresh weight).

^b Determined by the phenol-H₂SO₄ method, with glucose used for standard.

^c ND, not determined.

fractions. The soluble fraction was made 5% with respect to TCA and centrifuged to remove precipitated protein and cytoplasmic materials. From the supernatant, a water-soluble polysaccharide fraction, WSP-I, was prepared. The insoluble fraction from the buffer-homogenate was treated with LiCl to remove a fraction of wall-associated proteins, and solubilized material was recovered to give a water-soluble polysaccharide fraction, WSP-II. LiCl-treated cell walls were heated in a boiling water bath to inactivate residual wall-bound enzymes. Upon heating, the water-soluble polysaccharide fraction, WSP-III, was obtained. Neutral sugar composition analysis showed that WSP-I was rich in arabinose and galactose whereas WSP-II and WSP-III were primarily composed of glucose (Table I).

WSP-I, when subjected to chromatography on DEAE-Sephadex A-25 (phosphate type), gave an elution profile as shown in Figure 2. Tubes 3 to 5, 18 to 20, 21 to 26, and 27 to 40 were separately pooled and their neutral sugar composition was analyzed. Table II shows that the pooled fractions are all rich in arabinose and galactose residues. The unadsorbed fraction (tubes 3 to 5, WSP-I-1) and the adsorbed fraction (tubes 21 to 26, WSP-I-2) were separately subjected to gel filtration on Bio-Gel A-1.5m. WSP-I-1 yielded the three carbohydrate-containing peaks illustrated in Figure 3A. The major peak (tubes 79 to 91) was pooled, dialyzed and freeze-dried to give purified WSP-I-1. A Bio-Gel A-1.5m chromatogram of WSP-I-2 is presented in Figure 3B. The major peak (tubes 82–95) was pooled, dialyzed, and freeze-dried to give purified WSP-I-2. To assess the purity of purified WSP-

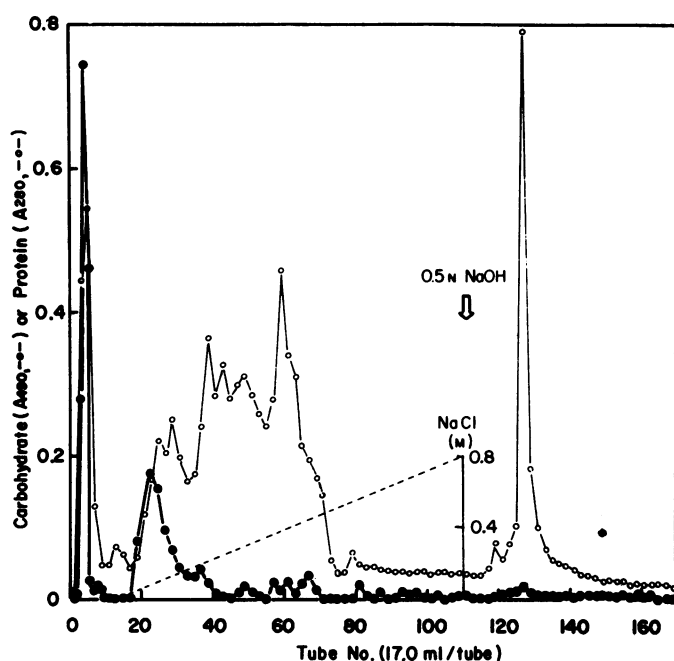


FIG. 2. DEAE-Sephadex A-25 chromatography of WSP-I. WSP-I (400 mg) in 10 ml of 20 mM Na-phosphate buffer (pH 6.0) was applied to a column (2.1 × 30 cm) of DEAE-Sephadex A-25 pre-equilibrated with the same buffer, and then the column was washed with the buffer. The adsorbed materials were eluted with a salt gradient (0–0.8 M NaCl in the Na-phosphate buffer) followed by elution with 0.5 N NaOH. Each tube was assayed for carbohydrate (A_{490}) and protein (A_{280}). Tubes 3 to 5, 18 to 20, 21 to 26, and 27 to 40 were separately combined, dialyzed against distilled H_2O , and freeze-dried. Combined fractions were individually assayed for their neutral sugar composition (Table II).

Table II. Yields and Neutral Sugar Composition of Fractions Obtained in Figure 2

Tube No. in Fig. 2	Yield	Neutral Sugar Composition				
		Ara	Xyl	Man	Gal	Glc
	mg			mol %		
3–5	62.0	36.3	3.6	2.0	50.4	7.7
18–20	7.0	42.2	2.3	0.4	49.1	6.0
21–26	30.5	37.2	6.4	Trace	56.4	Trace
27–40	23.0	29.8	7.0	Trace	61.1	2.2

I-1 and WSP-I-2, portions of WSP-I-1 and WSP-I-2 were separately rechromatographed on a calibrated Bio-Gel A-1.5m column. Purified WSP-I-1 and WSP-I-2 gave a single symmetrical peak; the filtration pattern of the phenol- H_2SO_4 -positive material corresponds with that of the carbazole- H_2SO_4 -positive material (uronic acid) (Fig. 4). Table III shows the neutral sugar composition of various portions of the carbohydrate peaks of purified WSP-I-1. The ratios of all of the neutral sugar residues were almost the same in all of the fractions examined.

The purified WSP-I-1 and -2 are hereafter designated as arabinogalactan-1 (AG-1) and arabinogalactan-2 (AG-2).

General properties of AG-1. AG-1 had $[\alpha]_D^{25} = -56^\circ$ ($C = 0.5$, in H_2O) and was soluble in water. Sugar composition analysis showed that AG-1 consisted of arabinose, galactose, xylose, uronic acid, and glucose in the molar ratio of 37.1:55.8:3.0:4.1:trace. A trace of protein was detected in AG-1. Chromatography of AG-1 on a calibrated Bio-Gel A-1.5m column indicated an average mol wt of 1.2×10^5 by comparison to standard dextrans (Fig. 4A). These results are summarized in Table IV.

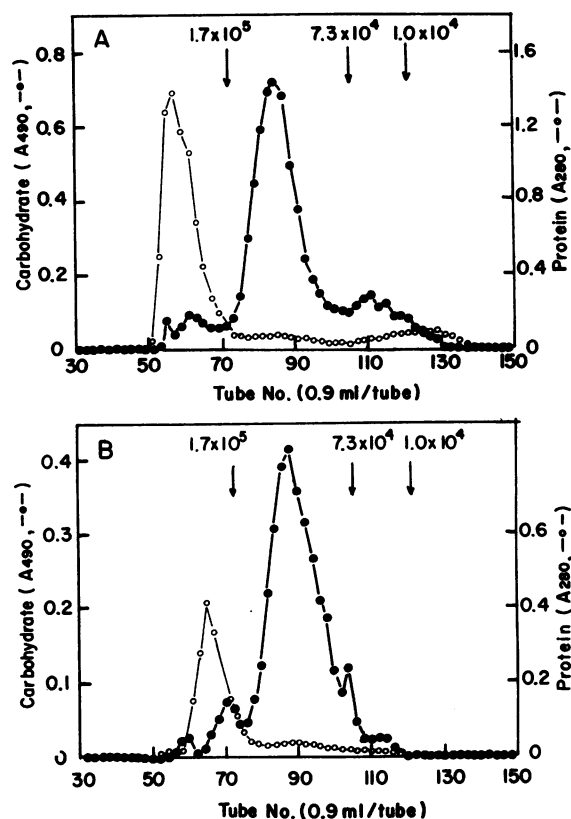


FIG. 3. Bio-Gel A-1.5m chromatography of WSP-I-1 and WSP-I-2 obtained in Figure 2. WSP-I-1 (30 mg) in 2 ml of 20 mM Na-phosphate buffer (pH 6.0) was applied to a column (2.2 × 52 cm) of Bio-Gel A-1.5m pre-equilibrated with the same buffer followed by filtration with the same buffer (A). Each tube was assayed for carbohydrate (A_{490}) and protein (A_{280}). Tubes 79 to 91 were combined, dialyzed against distilled H_2O , and freeze-dried to give purified WSP-I-1 (yield: 15 mg). WSP-I-2 (20 mg) was chromatographed under the same conditions (B), and 10 mg of purified WSP-I-2 was obtained. The arrows index the elution positions of dextrans of mol wt 1.7×10^5 , 7.0×10^4 , and 1.0×10^4 .

Glycosidic Linkage Composition of AG-1. AG-1 was methylated according to the procedure of Hakomori (9) as described by Sandford and Conrad (27). Two-thirds of the methylated AG-1 was treated with $LiAlH_4$ to reduce the uronic acid residues in AG-1 which had been esterified in the methylation reaction. One-half of the carboxyl-reduced methylated AG-1 was remethylated by the method of Hakomori to methylate the derived primary hydroxyl group. The methylated AG-1, the carboxyl-reduced methylated AG-1, and the methylated, carboxyl-reduced methylated AG-1 were separately hydrolyzed with acid; the resulting methylated monosaccharides were identified as their alditol acetates by GLC. The identities of all the *O*-methylglycitols were confirmed by combined GLC-MS. A gas chromatogram of the methylated, carboxyl-reduced methylated AG-1 is presented in Figure 5. A summary of all of the identified derivatives present in three kinds of samples is shown in columns 1, 2 and 3 of Table V.

The relative abundance of 2,3,5-tri-*O*-methyl-L-arabinose and 2,4-di-*O*-methyl-D-galactose in the methylated AG-1 suggests that AG-1 is either a polysaccharide having a linear backbone chain of (1 → 3)-D-galactan residues most of which are substituted in the 6-position by arabinose residues or a polysaccharide having a (1 → 6)-D-galactan backbone chain most of which are branched through O-3 by arabinose residues, or a polysaccharide which contains interior chains of D-galactose residues mutually joined by (1 → 3) and (1 → 6) linkages which carry outer chains

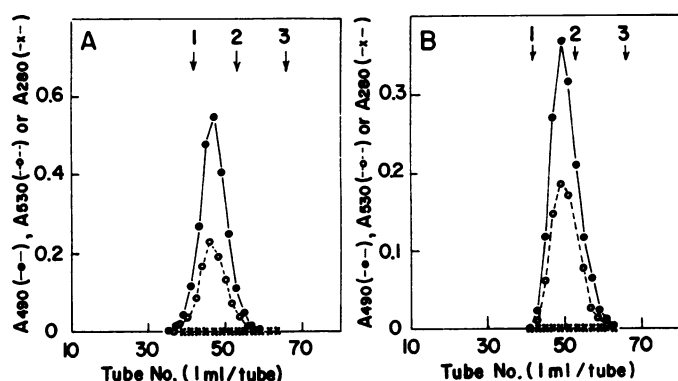


FIG. 4. Bio-Gel A-1.5m chromatography of purified WSP-I-1 and WSP-I-2 obtained in Figure 3. A, Purified WSP-I-1 (1.1 mg) in 0.3 ml of 20 mM Na-phosphate buffer containing 0.1% NaN₃ (pH 6.0) was applied to a column (1.3 × 65 cm) of Bio-Gel A-1.5m pre-equilibrated with the same buffer followed by filtration with the same buffer. Each tube was assayed for carbohydrate (A₄₉₀), uronic acid (A₅₃₀), and protein (A₂₈₀). Assay for uronic acid was carried out using a dialysate of each fraction against distilled H₂O because the buffer inhibited detection of uronic acid. Tubes 42, 44, 46, 48, 50, and 52 were individually assayed for their neutral sugar composition (Table III). B, Purified WSP-I-2 (0.8 mg) in 0.3 ml of 20 mM Na-phosphate buffer containing 0.1% NaN₃ (pH 6.0) was chromatographed under the same condition. Arrows 1, 2, and 3 index the elution positions of dextrans of mol wt 1.7×10^5 , 7.0×10^4 , and 1.0×10^4 , respectively.

Table III. Comparison of Neutral Sugar Composition of Selected Tubes in Peak of Purified WSP-I-1 Eluting from Bio-Gel A-1.5m

Tube in Fig. 4A	Neutral Sugar Composition			
	Ara	Xyl	Gal	Glc
	mol %			
42	44.2	4.2	51.5	Trace
44	42.2	2.3	55.6	Trace
46	38.1	1.3	60.6	Trace
48	44.0	0.2	53.9	Trace
50	45.1	2.5	52.4	Trace
52	41.4	3.3	55.2	Trace

Table IV. Analysis and Properties of Isolated Arabinogalactans

	Arabinogalactan-1		Arabinogalactan-2	
	Mol Wt	1.2 × 10 ⁵	Mol Wt	1.1 × 10 ⁵
	[α] _D	-56° (C = 0.5 in H ₂ O)		ND ^a
Composition	wt %	mol %	wt %	mol %
Carbohydrate	100		99.8	
Ara	33.0	37.1	31.1	35.4
Xyl	2.7	3.0	1.4	1.6
Gal	59.6	55.8	56.8	53.9
Glu	Trace	Trace	Trace	Trace
Uronic acid ^b	4.7	4.1	10.4	9.2
Protein	Trace		0.2	

^a ND, not determined.

^b Determined by the carbazol-H₂SO₄ method, with glucuronic acid used for standard.

terminated by arabinose in addition to some galactose residues. Furthermore, the detection of 2,3,4-tri-*O*-methyl-D-glucose in the carboxyl-reduced methylated AG-1 and the appearance of 2,3,4,6-tetra-*O*-methyl-D-glucose and the concomitant disappearance of 2,3,4-tri-*O*-methyl-D-glucose in the methylated, carboxyl-reduced methylated AG-1 indicate that uronic acid resi-

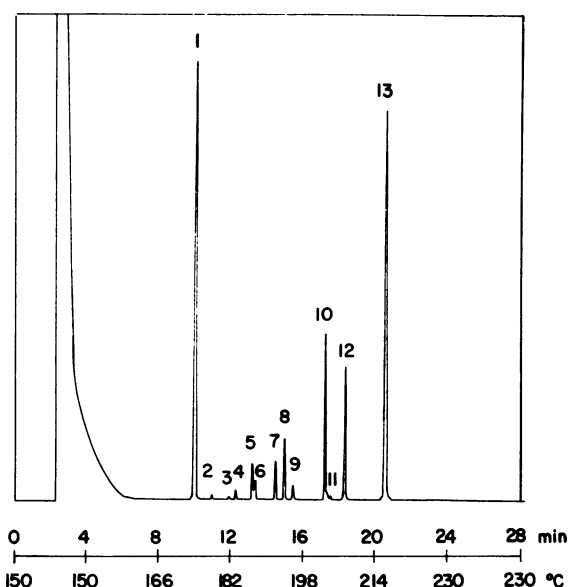


FIG. 5. Gas-liquid chromatogram of the partially methylated alditol acetates obtained from the acid hydrolysate of the methylated, carboxyl-reduced methylated AG-1. The sample was analyzed on column c at a helium flow rate of 1 ml/min. The 0.1-μl sample was injected at a column temperature of 150°C, and after 4 min the temperature was raised at 4°C/min to 230°C. Peaks are numbered from 1 to 13, and peak numbers are identified and peak areas quantitated in Table V.

dues in AG-1 are glucuronic acid which is present at the nonreducing terminal positions of AG-1.

Partial Acid Hydrolysis of AG-1 and Analyses of the Hydrolysate. AG-1 was hydrolyzed with weak acid and the product was resolved into higher (AG-1-a, 59.6% of recovered carbohydrate) and lower mol wt fractions (AG-1-b, 40.4%) by the methanol fractional precipitation method. Paper chromatography of AG-1-b revealed that it was composed mainly of arabinose and oligosaccharide(s) which remained at the origin. The latter was not further studied. Neutral sugar analysis showed that AG-1-a consisted of galactose and arabinose in the molar ratio of 98.2:1.8. GLC of the alditol acetates obtained from a hydrolysate of the methylated AG-1-a showed that the molar ratio of 2,3,4-tri-*O*-methyl-D-galactose, 2,4-di-*O*-methyl-D-galactose, 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,4,6-tri-*O*-methyl-D-galactose, and 2,3,5-tri-*O*-methyl-L-arabinose was approximately 36:9:8:4:1 (column 4 of Table V). These methylation studies of AG-1 indicate that most of the arabinose residues are attached to O-3 of (1 → 6)-linked galactopyranose residues.

Acid hydrolysis of AG-1-a followed by fractionation of the hydrolysate by gel filtration on Bio-Gel P-2 afforded four carbohydrate fractions, AG-1-a-i to -iv (Fig. 6). Fractions AG-1-a-ii to -iv, corresponding to the elution positions of tri-, di-, and monosaccharides, were not further studied. Fraction AG-1-a-i which eluted near the void volume of the P-2 column accounted for about 9.2% of total peak area in Figure 6. AG-1-a-i consisted of neutral and acidic sugars in the ratio of approximately 1:1.4 as determined by the phenol-H₂SO₄ method (8) and the carbazole-H₂SO₄ method (3). The neutral sugar residue was mostly galactose with a trace of xylose and glucose. The degree of polymerization of AG-1-a-i was 1.8 which was determined on the basis of reducing sugar/total sugar. AG-1-a-i was methylated, reduced, and methylated in a manner almost identical with that described for AG-1. The fully methylated neutral sugar was hydrolyzed and converted into corresponding alditol acetates. Gas chromatographic analysis of the partially methylated alditol acetates showed the presence of nonreducing terminal glucose

Table V. Glycosidic Linkage Analyses of Polysaccharides

Methylated Sugar ^a	Peak No. in Fig. 5	Deduced Glycosidic Linkage ^b	Polysaccharide ^c				
			1	2	3	4	5
2,3,5-Me ₃ -Ara	1	T-Ara	34.6	31.4	28.7	1.7	38.1
2,3,4-Me ₃ -Xyl	2	T-Xyl	Trace	0.2	0.2	0	Trace
3,5-Me ₂ -Ara	3	2-Ara	0.7	Trace	Trace	0	1.2
2,5-Me ₂ -Ara	4	3-Ara	0.5	1.7	1.0	0	0.6
2,3-Me ₂ -Ara	5	5-Ara	2.2	3.2	2.7	0	1.9
2,3-Me ₂ -Xyl	6	4-Xyl	0.8	1.8	2.7	0	1.2
2,3,4,6-Me ₄ -Glc	7	T-Glc	0	0	2.2	0	0
2,3,4,6-Me ₄ -Gal	8	T-Gal	2.9	2.6	3.7	14.2	4.2
2- and/or 3-Me ₁ -Xyl	9	3,4- and/or 2,4-Xyl	1.4	0.6	0.6	0	1.9
2,4,6-Me ₃ -Gal	10	3-Gal	10.9	10.8	13.4	7.7	11.1
2,3,4-Me ₃ -Glc	11	6-Glc	0	2.3	Trace		0
2,3,4-Me ₃ -Gal	12	6-Gal	3.9	7.3	9.7	61.4	61.4
2,4-Me ₂ -Gal	13	3,6-Gal	42.2	38.3	35.0	15.1	34.1

^a 2,3,5-Me₃-Ara = 2,3,5-tri-*O*-methyl-L-arabinose, etc.

^b The glycosidic linkages of each sugar derivative are indicated by numerical prefixes: thus, 3,6-Gal indicates that sugars are glycosidically linked in the polysaccharides to the 3- and 6-carbons of these galactosyl residues. Nonreducing terminal residues are indicated by T-.

^c 1: the methylated arabinogalactan-1; 2: the carboxyl-reduced methylated arabinogalactan-1; 3: the methylated, carboxyl-reduced methylated arabinogalactan-1; 4: the methylated arabinogalactan-1-a; 5: the methylated arabinogalactan-2.

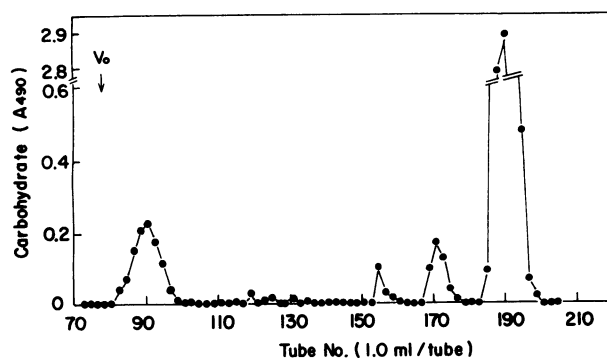


FIG. 6. Bio-Gel P-2 chromatography of the acid hydrolysate of AG-1-a. Acid-hydrolysate of AG-1-a (2.3 mg) in 2 ml of 0.1 M NaCl was applied to a column (1.5 × 150 cm) of Bio-Gel P-2 followed by filtration with water at 50°C. Each tube was assayed for carbohydrate (A_{490}). Tubes 85 to 96, 153 to 161, 167 to 179, and 183 to 201 were separately combined to give fractions AG-1-a-i, -ii, -iii, and -iv.

and 6-linked galactose residues in the approximate ratio of 1:1. From these results, AG-1-a-i was considered to be mostly composed of glucuronosyl-(1 → 6)-galactose.

Characterization of AG-2. AG-2 was soluble in water. Sugar composition analysis showed that AG-2 consisted of arabinose, galactose, xylose, uronic acid, and glucose in the molar ratio of 35.4:53.9:1.6:9.2:trace. A small quantity of protein (0.2%) was present in AG-2. An average mol wt of AG-2 was estimated to be 1.1×10^5 by using a calibrated Bio-Gel A-1.5m column (Fig. 4B). Glycosidic linkage analysis by GLC of the alditol acetates obtained from the acid hydrolysate of the methylated AG-2 showed that glycosidic linkage composition is very similar to that of AG-1 (column 5 of Table V).

AG-2 was hydrolyzed with acid and the hydrolysate was chromatographed on a column of Bio-Gel P-2. Figure 7 shows the elution profile. Fraction AG-2-b, which eluted near the void volume of the P-2 column and accounted for about 12% of the total peak area in Figure 7, was subjected to structural analysis. AG-2-b consisted of neutral and acidic sugars in the ratio of approximately 1:1.4. The neutral sugar consisted mostly of galactose but small amounts of xylose, arabinose, and glucose were

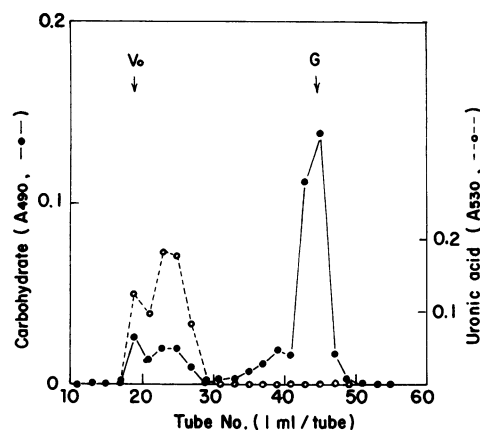


FIG. 7. Bio-Gel P-2 chromatography of the acid hydrolysate of AG-2. AG-2 (1.4 mg) hydrolyzed under reflux with 1 ml of 0.1 N HCl in a boiling water bath for 3 h. After hydrolysis, the solution was neutralized with 1 N NaOH. The resultant solution was applied to a column (1.2 × 60 cm) of Bio-Gel P-2 followed by filtration with water at 50°C. Each tube was assayed for carbohydrate (A_{490}) and uronic acid (A_{530}). Tubes 16 to 21, 22 to 30, 31 to 36, 37 to 41, and 42 to 48 were separately pooled to give fractions AG-2-a, -b, -c, -d, and -e.

detected. Methylation analysis, which was conducted in a manner almost identical with that described for AG-1-a-i, showed that 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-galactose were prominent structural units in the fully methylated AG-2-b. The ratio of the derived nonreducing terminal glucose and 6-linked galactose residues was approximately 1:1. Therefore, the major oligosaccharide in AG-2-b is proposed to have the structure: glucuronopyranosyl-(1 → 6)-galactose.

DISCUSSION

The structures of plant arabinogalactans and arabinogalactan-proteins have been extensively investigated (1, 5, 29 and references cited therein). The polysaccharides are grouped into three structural types: type I by Aspinall (arabino-4-galactan), type II by Aspinall (arabino-3,6-galactan), and others. In fact, there have

been several reports on such polysaccharides and polysaccharide-proteins in monocot tissues (5). However, no detailed information has been available concerning the polysaccharide in growing monocots except for a paper indicating the presence of arabinose- and galactose-rich polysaccharides in the *Avena* coleoptile (31).

Arabino-3,6-galactans (AG-1 and AG-2) isolated from *Zea* shoots contain a trace and small amounts of proteins, respectively. It is not known whether the protein in the preparations is an impurity or is chemically bound to the polysaccharide. If the latter is the case, the protein may be hydroxyproline-rich and associated with the polysaccharide as described previously (5).

Methylation analysis indicates that about 90% of total arabinose residues are present as nonreducing terminal residues in AG-1 or AG-2 and attached to O-3 of (1 → 6)-linked galactopyranosyl residues. Partial acid hydrolysis removed most of the arabinose residues, leaving the galactan to which the arabinose residues had been attached. It is clear that the liberation is due to ease of the acid hydrolysis of the arabinofuranosidic linkage. The detection of 2-, 3-, and 5-linked arabinose residues suggests several potential side chains present in AG-1 and AG-2, for example, arabinofuranosyl-(1 → 2, 3 or 5)-arabinose and galactopyranosyl-(1 → 2, 3 or 5)-arabinose.

In some arabino-3,6-galactans, uronic acid has been found, and the content and type of uronic acid has been recorded. However, no uronic acid has been reported in arabino-3,6-galactan from *Triticum aestivum* seed, *Oryza sativa* bran, *Phyllostachys edulis* shoots, or suspension-cultured *Lolium multiflorum* cells which are all grasses (5 and references cited therein). Glycosidic linkage analysis of the carboxyl-reduced methylated AG-1 and the methylated carboxyl-reduced methylated AG-1 indicates that uronide present in AG-1 is glucuronic acid in nonreducing terminal positions of the polysaccharides. Isolation of glucuronosyl-(1 → 6)-galactose from partially degraded AG-1 (AG-1-a) or from AG-2 confirms glucuronosyl residues are linked to O-6 of galactose residues in AG-1 or AG-2. It is not known whether the glucuronosyl residues are esterified by methyl groups.

The 4-linked and 3,4- and/or 2,4-linked xylose residues found in AG-1 and AG-2 may be derived from an arabinoxylan-like polysaccharide which co-purified with arabino-3,6-galactan. The amount of arabinoxylan-like polysaccharide in AG-1 or AG-2 can be estimated by considering that all of the 4-linked and the 3,4- and/or 2,4-linked xylose residues are probably in arabinoxylan and that the 3,4- and/or 2,4-linked xylose residues must also be accompanied by an equal number of terminal residues, including the terminal arabinose, plus other termini probably including glucuronic acid. Therefore, these arabinoxylan components add up to a maximum of 4 to 5% of AG-1 or AG-2. However, the possibility still remains that such arabinoxylan-like polysaccharide may be linked to arabino-3,6-galactans by covalent linkage. This uncertainty will remain until complete investigation of such xylose-containing portion is undertaken. Another possibility, not denied by these results, is that such xylose residues are present as substituents of the galactan framework of AG-1 or AG-2.

From the results of methylation studies, about 20% of the galactose residues in AG-1 constitute a (1 → 3)-linked galactan sequence and approximately 60% of the galactan in AG-1 is (1 → 6)-linked. Furthermore, about 15% of the galactose residues in AG-1 are substituted by galactose in the 3- and 6-positions, thereby constituting branch points of the galactan framework. The remainder (5%) of the galactose residues in AG-1 are located at nonreducing terminal positions. About 85% of the (1 → 6)-linked galactosyl sequence is substituted mostly by single arabinose residues in the 3-position.

The investigation shows that the arabinogalactans from *Zea* shoots belong to the type II arabino-3,6-galactans according to

Aspinall. Arabino-3,6-galactans or arabino-3,6-galactan proteins which are not likely to be structural components of the wall have been isolated from various kinds of monocotyledon tissues (5, 7). Although no arabino-3,6-galactan has been isolated from differentiated primary cell walls of monocots, the presence of 3-, 6-, and 3,6-linked galactosyl residues has been shown in the cell walls of several suspension-cultured monocotyledons (4). Glycosidic linkage analysis of purified *Zea* shoot cell walls obtained from "cell walls" in Figure 1 has shown the presence of 3-, 4-, and 6-linked and nonreducing terminal galactosyl residues in the *Zea* cell walls. The concentration of 3- and 6-linked galactosyl residues, which seem to be derived from arabino-3,6-galactan, is lower than that of 4-linked and nonreducing terminal galactosyl residues which are components of 4-linked galactan and glucuronoarabinoxylan, respectively (unpublished data). It is not certain whether glucuronic acid-containing arabino-3,6-galactans isolated from *Zea* shoots are intermediates destined for deposition in the cell walls or components loosely associated with cell walls, or if they may have other roles. The questions related to whether or not these polysaccharides, which resemble structural components of dicot primary cell walls, are components of *Zea* shoot cell walls, will remain until detailed analysis of *Zea* shoot cell walls is complete.

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